The Spiro Intermediate Proposed for Biosynthesis of the Natural Porphyrins: Synthesis and Properties of Its Macrocycle

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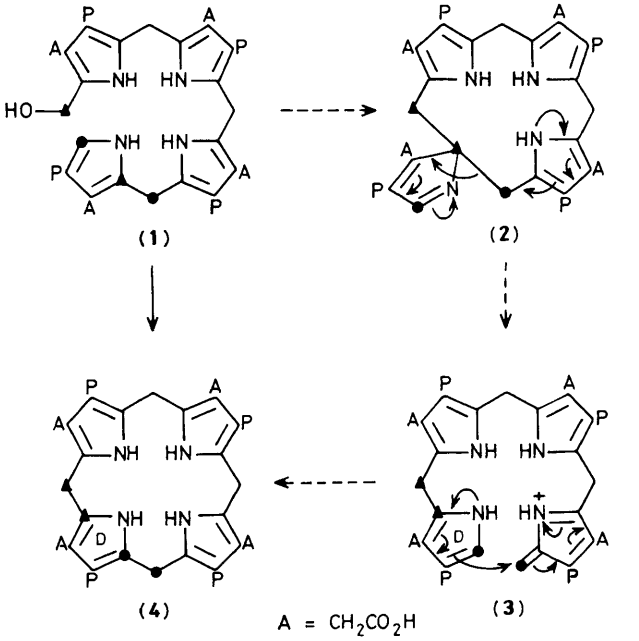
Two structures have been synthesised containing the novel macrocycle on which is based the hypothetical spiro-intermediate for biosynthesis of the natural porphyrins; the strongly puckered conformation of the macrocycle is shown by *X*-ray structure analysis.

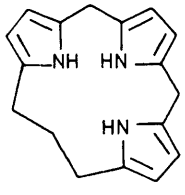
All the pigments of life (*e.g.* haem, chlorophyll, and vitamin B_{12}) are derived in Nature from one macrocycle, uroporphyrinogen-III (**4**), shortened to uro'gen-III. Extensive studies have been made of the biosynthesis of this substance and the results have been reviewed.[1] The final stages in the biosynthesis are catalysed by the enzyme cosynthetase which ring-closes the hydroxymethylbilane (**1**) with intramolecular rearrangement of ring-D to afford uro'gen-III (**4**). The symbols ● and ▲ on structures (**1**) and (**4**) mark the ^{13}C-labelling experiments, one with ^{13}C at ● and a second with ^{13}C at ▲, which revealed[2] the changes in bonding as (**1**) is converted into (**4**).

These results and many others[1] eliminated all but two of the *ca.* 25 hypothetical schemes in the literature which sought to explain the formation of uro'gen-III (**4**). One of the remaining two is referred to as the external methylene scheme;[3] this will not be considered further here. The other[4] invokes the intermediacy of a spiro-pyrrolenine (**2**) (see also comments below); the illustrated fragmentation *via* (**3**) could plausibly account for the conversion of the bilane (**1**) into uro'gen-III (**4**). That the fragmentation–recombination process (**2**) → (**3**) → (**4**) involved in this hypothesis is both feasible and facile has been demonstrated by the synthesis of a suitable 2-pyrrolylmethylpyrrolenine[5] which underwent ready rearrangement.

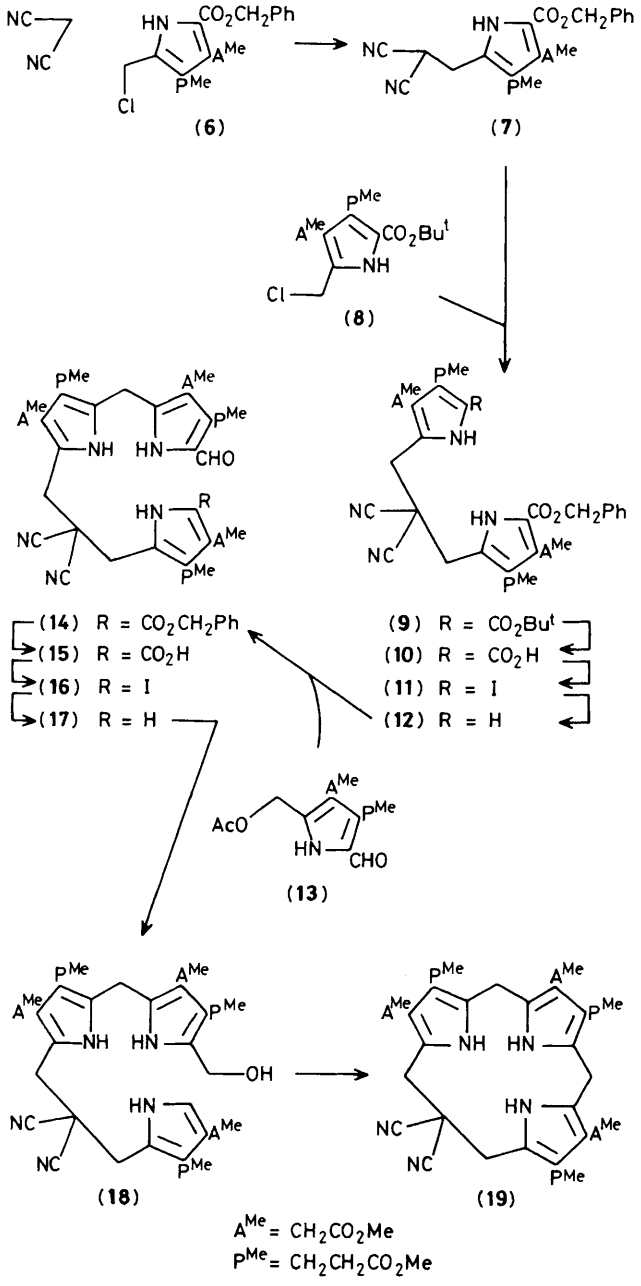
Structure (**2**) for the putative spiro-intermediate has been written for decades but no example of this type of macrocycle is known; the parent ring system is (**5**). Doubts have been raised as to whether structure (**2**) is sterically possible; indeed, the original proposers of the spiro idea[4] did not use structure (**2**) but a triply *C*-protonated form of it arguing that the increased flexibility from additional sp^3 carbons was important for macrocycle formation. A study was therefore undertaken of the synthesis of substances based on system (**5**).

The first synthetic target was the dinitrile (**19**) and the route started with alkylation of malononitrile by the chloromethyl-





(**5**)



pyrrole[6] (**6**) using Hünig's base to yield the dinitrile (**7**). The same conditions allowed introduction of a second pyrrolylmethyl group, from pyrrole[7] (**8**), to yield the differentially protected system (**9**). Trifluoroacetic acid cleaved the t-butyl ester and then iodination of the acid (**10**) gave (**11**) from which the iodide residue was removed by catalytic hydrogenation (Pd–C with NaOAc) yielding the pyrrole (**12**) with a free α-position. This was alkylated using the acetoxy aldehyde[8] (**13**) to give (**14**), a reaction requiring controlled catalysis by tin(IV) chloride in methylene chloride for success.

Removal of the benzyl group from (**14**) by hydrogenolysis yielded the acid (**15**) which was converted into (**17**) [iodination to form (**16**) then hydrogenation (Pd–C, NaOAc)]. Borohydride reduced the aldehyde (**17**) to the alcohol (**18**) and this on treatment with toluene-*p*-sulphonic acid in methylene chloride cyclised to give one product (**19**), 25% yield over last 2 stages, found M^+ *m/z* 789.3211, $C_{40}H_{47}N_5O_{12}$ requires 789.3221. It was stable and crystalline though the crystals

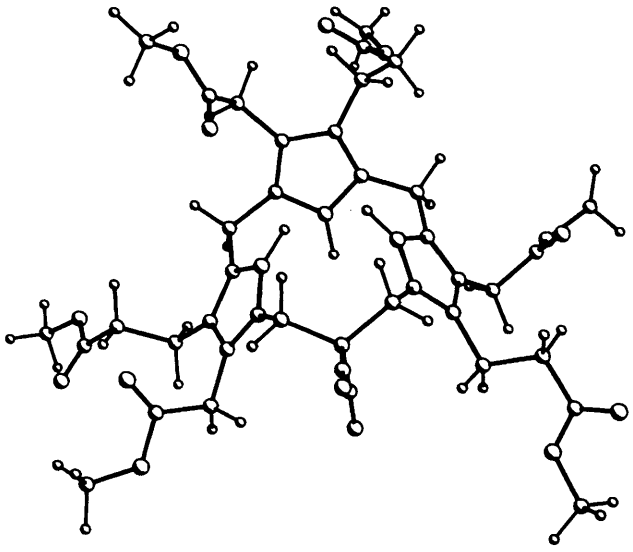
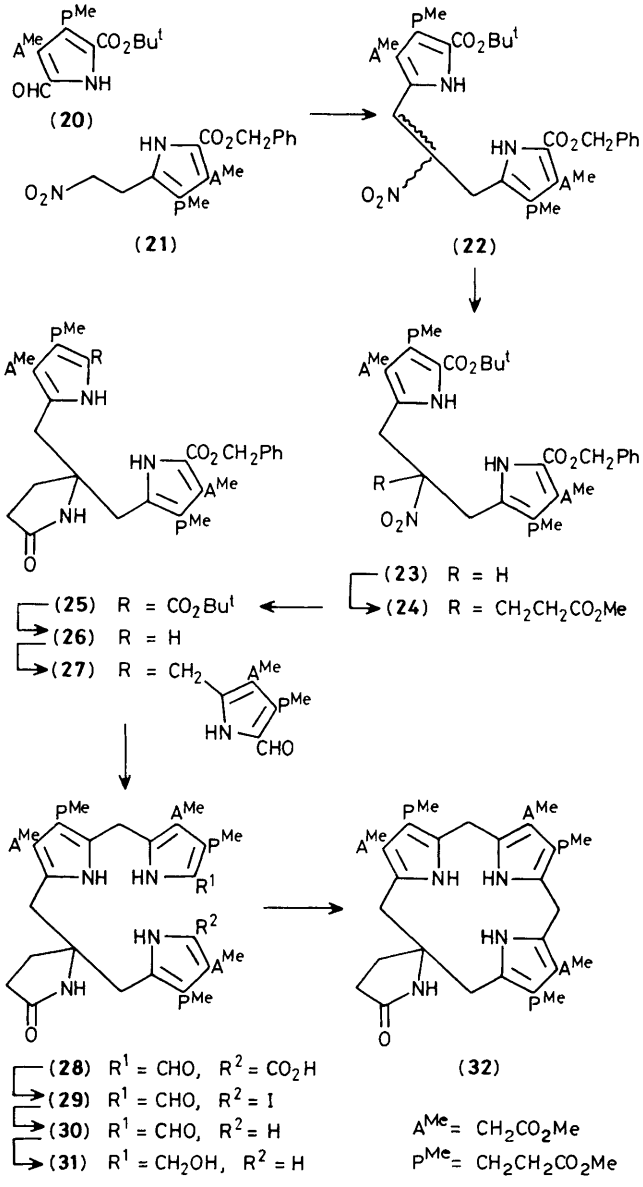


Figure 1. The molecular structure of macrocycle (**19**).



powdered as solvent of crystallisation was removed. An *X*-ray structure determination was carried out on a crystal of (**19**) suspended in its mother liquor.† Figure 1 shows the result which confirmed that the desired macrocycle had been formed and gave a striking view of the molecular conformation. The macrocycle is strongly puckered with the pyrrolic NH atoms of the two pyrroles adjacent to the quaternary centre pointing in the opposite direction to the NH of the third pyrrole.

Our next aim was to construct a molecule even more closely related to that of the proposed spiro-intermediate (**2**); structure (**32**) was selected. Condensation of the aldehyde (**20**) with the nitroalkane[9] (**21**) using methylamine–acetic acid afforded a 2:1 mixture of the *E* and *Z* alkenes (**22**). These were reduced by borohydride to the same nitroalkane (**23**) whose anion (generated by Triton B) underwent Michael addition to methyl acrylate to yield the quaternary system (**24**). Reduction of the nitro group in (**24**) with zinc–HOAc led directly to the lactam (**25**). The substitution pattern on the two pyrrolic rings of this product (**25**) exactly matches that for the dipyrrole (**9**) built above during the synthesis of the dinitrile (**19**). Accordingly, strictly analogous synthetic steps could be used for the rest of the synthesis, *viz.* (**25**) → (**26**) → (**27**) → (**28**) → (**29**) → (**30**) → (**31**) → (**32**). The final stage of ring-closure to generate the macrocycle had to be done in an oxygen-free glove box otherwise very ready oxidation of the material occurred [*cf.* the stable dinitrile (**19**) above which has two additional strong electron-withdrawing groups]. Interestingly, *two* separable spiro-products were obtained (36% yield over 2 stages), both $C_{41}H_{52}N_4O_{13}$ by accurate mass measurement (error for both < 4 p.p.m.) and the ^{1}H n.m.r. spectrum (400 MHz) of each was fully consistent with structure (**32**). On a space-filling model of structure (**32**), the amide NH occupies space over the centre of the macrocycle. This makes it difficult to convert one puckered conformation into another and leads to the view that the two isomers above are different locked conformers. It should be noted that the hypothetical spiro-intermediate (**2**) lacks this NH group.

The foregoing work provides the first examples of macrocycles based on the parent (**5**), which are of great biosynthetic interest and turn out to be reasonably stable. Thus both the pyrrolylmethylpyrrolenine[5] and macrocyclic portions of the putative spiro-intermediate (**2**) are now available synthetically.

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† *Crystal data:* (**19**) $C_{40}H_{47}N_5O_{12}\cdot C_6H_6$, M = 867.94, triclinic, space group $P\bar{1}$ (No. 2), a = 11.834(1), b = 14.632(1), c = 14.675(2) Å, α = 64.17(1), β = 87.73(1), γ = 82.19(1)°, U = 2265.4 Å^3, Z = 2, D_c = 1.27 g cm^{-3}, $F(000)$ = 892, $\lambda(\text{Mo}-K_\alpha)$ = 0.71069 Å, $\mu(\text{Mo}-K_\alpha)$ = 0.54 cm^{-1}, Current R = 0.121 and R_w = 0.120 for 2681 unique diffractometer data with $5 < 2\theta < 42.5°$ and $F > 4\sigma(F)$; all non-hydrogen atoms except those in the inner ring were refined anisotropically; H atoms were placed in idealised positions and each type was refined with a common isotropic temperature factor; the benzene solvent molecule was treated as a rigid group. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

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